OPEN ACCESS

POSITION STATEMENT

Cancer Variant Interpretation Group UK (CanVIG-UK): an exemplar national subspecialty multidisciplinary network

Alice Garrett ,[1] Alison Callaway,[2,3] Miranda Durkie,[4] Cankut Cubuk,[1,5] Mary Alikian,[1] George J Burghel ,[6] Rachel Robinson,[7] Louise Izatt,[8] Sabrina Talukdar,[9] Lucy Side,[10] Treena Cranston,[11] Sheila Palmer-Smith,[12] Diana Baralle,[13] Ian R Berry ,[7] James Drummond,[14] Andrew J Wallace,[6] Gail Norbury,[15] Diana M Eccles ,[13] Sian Ellard,[16] Fiona Lalloo,[6] D Gareth Evans ,[6,17] Emma Woodward,[6,17] Marc Tischkowitz,[18] Helen Hanson,[1,9] Clare Turnbull ,[1,19] On behalf of the CanVIG-UK

► Additional material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/jmedgenet-2019-106759).

For numbered affiliations see end of article.

Correspondence to
Dr Clare Turnbull, Division of Genetics and Epidemiology, Institute of Cancer Research, Sutton SM2 5NG, UK; clare.turnbull@icr.ac.uk

AG, AC and MD contributed equally.

Received 4 December 2019
Revised 6 February 2020
Accepted 7 February 2020

To cite: Garrett A, Callaway A, Durkie M, *et al*. *J Med Genet* Epub ahead of print: [please include Day Month Year]. doi:10.1136/jmedgenet-2019-106759

ABSTRACT

Advances in technology have led to a massive expansion in the capacity for genomic analysis, with a commensurate fall in costs. The clinical indications for genomic testing have evolved markedly; the volume of clinical sequencing has increased dramatically; and the range of clinical professionals involved in the process has broadened. There is general acceptance that our early dichotomous paradigms of variants being pathogenic–high risk and benign–no risk are overly simplistic. There is increasing recognition that the clinical interpretation of genomic data requires significant expertise in disease–gene-variant associations specific to each disease area. Inaccurate interpretation can lead to clinical mismanagement, inconsistent information within families and misdirection of resources. It is for this reason that 'national subspecialist multidisciplinary meetings' (MDMs) for genomic interpretation have been articulated as key for the new NHS Genomic Medicine Service, of which Cancer Variant Interpretation Group UK (CanVIG-UK) is an early exemplar. CanVIG-UK was established in 2017 and now has >100 UK members, including at least one clinical diagnostic scientist and one clinical cancer geneticist from each of the 25 regional molecular genetics laboratories of the UK and Ireland. Through CanVIG-UK, we have established national consensus around variant interpretation for cancer susceptibility genes via monthly national teleconferenced MDMs and collaborative data sharing using a secure online portal. We describe here the activities of CanVIG-UK, including exemplar outputs and feedback from the membership.

BACKGROUND

Clinical utility of cancer susceptibility genes (CSGs)

Analysis of germline (constitutional) variants in CSGs constitutes approximately one-quarter of activity in NHS Molecular Diagnostic Laboratories in England.[1] Following identification of a pathogenic variant (PV) in a CSG, incidence of/mortality from future cancers may be mitigated via (1) risk-reducing surgery (eg, mastectomy, gastrectomy, salpingo-ophorectomy and colectomy); (2) chemoprevention; (3) intensive screening; and (4) lifestyle modification.[2] Family members negative for the familial CSG-PV can be spared anxiety and unnecessary screening. Many CSGs are associated with a pattern of cancer risk that is late-onset, variably penetrant and of autosomal dominant inheritance. PV-positive family members identified via cascade screening are often distributed across disparate genomics services.

Erroneous interpretation of CSG variant pathogenicity can therefore result in (1) discordant management within families, (2) serious clinical consequences for individuals and (3) misdirection at population level of resources for screening and prevention.[3–5] Increasingly, CSG-PVs are used as predictive biomarkers to inform cancer therapy. For all these reasons, robust, rapid, accurate variant analysis and interpretation of disease risk are critical to effective delivery of germline cancer genetics and improving outcomes for patients.

Evolving landscape of variant interpretation in germline cancer genetics

In the late 1990s, within a few years of identification of the relevant genes, laboratory analysis of CSGs became available in the UK via family cancer clinics.[2] If the cancer phenotype ascribed to the gene matched that found in the proband/family under study, with little additional evidence, a rare variant would often be labelled as pathogenic and thus causative.[6] Subsequent large-scale population sequencing studies have revealed the degree of innocuous variation present in the human genome (and indeed in disease-associated genes) and 'downgrading' of many erroneously labelled PVs has been required.[7] An era of caution followed, with much greater recourse to labelling of variants as 'variants of uncertain significance' (VUS/VOUS). However, lack of systems for sharing new evidence has meant that many families have spent years in limbo with their 'VUS', even when data had long been available by which classification of their variant could be downgraded or upgraded.

Sharing of clinical variant data was somewhat improved with the advent of locus-specific databases (LSDs), such as Breast Cancer Information Core and Leiden Open Variant Databases.[8–11] However, the curation of clinical and molecular data in LSDs often remains suboptimal, with (1) erroneous nomenclature, (2) duplication of entries and (3) use of differing classification systems resulting in contradictory assignations.[12]

Using Myriad Genetics data from ~70 000 genetic tests for hereditary breast and ovarian cancer, in 2007, Easton and colleagues published a landmark multifactorial analysis through which 'odds of causality' were mathematically generated for 1433 variants using clinical, pedigree and allelic data.[13] In 2008, International Agency for Research on Cancer (IARC) collaborators published the first formal five point variant interpretation system for CSGs, which included numeric thresholds for the probability of pathogenicity.[14] Expert cancer susceptibility consortia such as the Evidence-based Network for the Interpretation of Germline Mutant Alleles (ENIGMA) and the International Society for Gastrointestinal Hereditary Tumours (InSIGHT) further evolved these multifactorial variant classification systems to incorporate tumour phenotype and in silico predictions.[15–17] However, ENIGMA/InSIGHT approaches require statistical genetic–epidemiological analyses of large curated data series and are not reproducible by an individual diagnostic laboratory seeking to classify in a clinically relevant timescale a newly identified variant.

In 2015, the American College of Medical Geneticists (ACMG) published a variant interpretation framework enabling the combination by a diagnostic laboratory of disparate evidence sources for a newly identified genomic variant.[18] The ACMG framework has subsequently been further evolved under the auspices of ClinGen, including (1) specification for how it is applied to particular genes and/or diseases (including *TP53*, *CDH1* and *PTEN*); (2) deeper specification of particular criteria (eg, functional assays); and (3) exposition of the underpinning Bayesian model.[19–23]

Coordinated national UK approaches in variant interpretation

In 2016, with endorsement from NHS England and Health Education England, it was agreed formally by the UK Association of Clinical Genomic Science (UK-ACGS) to adopt the ACMG variant interpretation framework.[24 25] The UK-ACGS established national groups for rare disease, germline cancer genetics, cardiac disease and hypercholesterolaemia to develop and disseminate practice in the application of the ACMG variant interpretation framework.[24] In parallel was recognition within the NHS Genomic Medicine Service of the need for national subspecialist genomics MDMs.[26 27] In response to these dual recommendations, Cancer Variant Interpretation Group UK (CanVIG-UK) was initiated in 2017.

CANCER VARIANT INTERPRETATION GROUP UK

The purpose of CanVIG-UK is to advance outcomes for patients by improving the accuracy and consistency of interpretation of variants in CSGs across the UK clinical genetics and molecular diagnostic laboratory communities (hereafter termed the UK clinical-laboratory community). We aim to progress this goal by advancing six objectives (see box 1).

Creation of a national multidisciplinary professional network and regular forum

CanVIG-UK has grown to now include >100 members, incorporating clinical and laboratory representation from each of the 25 Molecular Diagnostic Laboratories and Clinical Genetics Services of the UK (NHS) and Ireland (see collaborators). This group comprises roughly equal proportions of clinical scientists and clinical geneticists, with two-thirds working exclusively or predominantly in cancer genetics (figure 1):

- The monthly teleconferenced MDM provides a *forum* to which problematic variants/cases are submitted. The variants submitted to the monthly variant surgery are circulated 1 week in advance. CanVIG-UK members are asked (1) to ascertain whether additional cases and/or laboratory data exist locally and (2) to undertake local, independent classification of the variant. The relevant clinical and laboratory data are presented by the nominating laboratory. This is followed by input of any additional information by the broader CanVIG-UK group and a discussion regarding the legitimacy of the ACMG criteria awarded. Following this discussion and an online postdiscussion poll, a consensus CanVIG classification is generated (see online supplementary table 1). A detailed date-stamped CanVIG variant summary sheet is generated (see online supplementary appendix 2), which is circulated by email, uploaded to the CanVar-UK portal and submitted to ClinVar.
- The CanVIG-UK *network* is active throughout the month via the email forum, through which urgent queries can be debated and addressed.

Box 1 CanVIG Objectives

The purpose of Cancer Variant Interpretation Group UK (CanVIG-UK) is to advance outcomes for patients by improving the accuracy and consistency of interpretation of variants in Cancer Susceptibility genes across the UK clinical-laboratory community. We have six specific objectives:

1. Creation of a national multidisciplinary professional network and regular forum.
2. Training and education.
3. Detailed specification for germline cancer genetics of the UK-ACGS Best Practice Guidelines for Variant Interpretation.
4. Ratification of additional guidance in germline cancer genetics relevant to the UK clinical-laboratory community.
5. Development of an online platform to facilitate information sharing and variant interpretation within the UK clinical-laboratory community.
6. UK contribution to international variant interpretation endeavours.

Training and education

The discussion of cases at the MDM also provides valuable education for the clinical-laboratory community regarding application of the ACMG framework and the vagaries of the evidence sources used (see figure 2). Additionally, through CanVIG-UK, we have supported training of the broader UK genetics and oncology communities in variant interpretation for CSGs.

Detailed specification for germline cancer genetics of the UK-ACGS Best Practice Guidelines for Variant Interpretation

On behalf of the UK-ACGS, the rare disease variant interpretation group has generated and updates annually a highly detailed specification of the ACMG variant interpretation framework .[24] In cancer susceptibility, we typically observe variants relating to late-onset, common phenotypes. De novo and biallelic

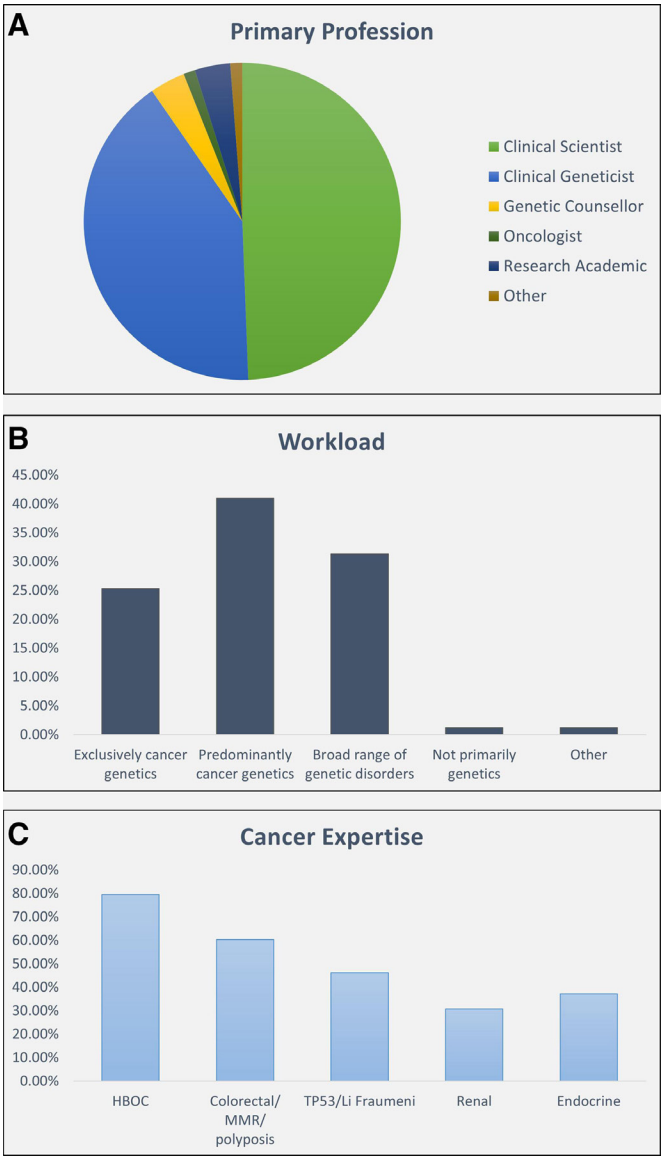

Figure 1 Overview of the CanVIG-UK membership profile (survey of CanVIG-UK members, performed on 29 October 2019, return rate 83/103 (81%). HBOC, hereditary breast and ovarian cancer; MMR, mismatch repair. CanVIG-UK, Cancer Variant Interpretation Group UK.

paradigms are infrequent. We are typically much more reliant on variant frequency from case series and functional assays. Thus, an important remit for CanVIG-UK has been to develop a detailed specification of the UK-ACGS framework for these types of evidence to be used for CSG variant interpretation (see online supplementary appendix 1).

Ratification of additional guidance in germline cancer genetics relevant to the UK clinical-laboratory community

Historically, the first presentation to the family cancer clinic was typically an unaffected individual, concerned by a significant family history. Increasingly, genetic analysis is now performed as part of routine work-up at cancer diagnosis, either through analysis of a germline sample or through therapeutically motivated molecular analysis of the tumour. In both contexts, (1) focused testing of one or two genes has often been superseded by broad 'cancer panels' containing dozens or hundreds of genes; (2) patients may be unselected for family history; and (3) analysis and reporting in a tight time frame is typically required. A number of challenging issues have emerged, including

1. Categorisation and management of reduced penetrance variants in high-penetrance genes.
2. Variant interpretation and clinical management for moderate-penetrance genes.
3. Adaptation of variant interpretation and risk for different contexts of ascertainment.
4. Inference of germline findings from tumour-only sequencing.

While germane across genomics, consideration of these issues has become pressing within germline cancer genetics. Benefitting from its regular forum, multidisciplinary membership and alignment with both UK-ACGS and the UK Cancer Genetics Group (UK-CGG), we have used the CanVIG–UK monthly forum to evolve UK national multidisciplinary approaches on such issues (see online supplementary appendix 3).

Development of an online platform to facilitate information sharing and variant interpretation within the UK clinical-laboratory community

In germline cancer genetics, enrichment in cases (especially 'strong families') is one of the most valuable clinical observations indicating variant pathogenicity. However, to date, we have struggled to quantify such observations on account of (1) failure to aggregate national data from distributed laboratories and (2) lack of a robust denominator.

In a collaborative venture between Public Health England (PHE) and the national network of molecular diagnostic laboratories, data from molecular testing of CSGs have been submitted via a pseudonymisation portal to the secure National Cancer Registration and Analysis Service (NCRAS) data environment of PHE.[28] The national variant totals (numerator and denominator) are then shared by CanVIG-UK with the UK clinical-laboratory community via our online data system CanVar-UK (http://www.canvaruk.org/).

CanVar-UK provides additional annotations for 1 008 643 variants from 95 CSGs. It includes variant-level annotations from LSDs (case counts), functional assays, splicing assays and multifactorial analyses for selected genes. Accessible only to registered CanVIG-UK clinical-laboratory users is a community area for sharing non-identifiable variant-level data, such as local classifications, comments/notes, uploaded documents and results from local laboratory assays (eg, RNA analyses of potential splicing variants).

UK contribution to international variant interpretation endeavours

CanVIG-UK is an effective conduit between the UK clinical-laboratory germline cancer genetics community and relevant international variant interpretation endeavours in several regards:

- First, there is representation at the international ClinGen SVI group from the leadership of the UK-ACGS rare disease variant interpretation group. The regular crosstalk between leadership of the UK groups enables appraisal of the ClinGen SVI group of emerging analyses and activity within CanVIG-UK and the UK clinical-laboratory cancer genetics community.
- Second, multiple members of CanVIG-UK are members of gene-specific international endeavours such as ENIGMA, InSIGHT and ClinGen expert groups.
- Third, data generated by CanVIG-UK data have contributed to collaborative international consortia analyses, for

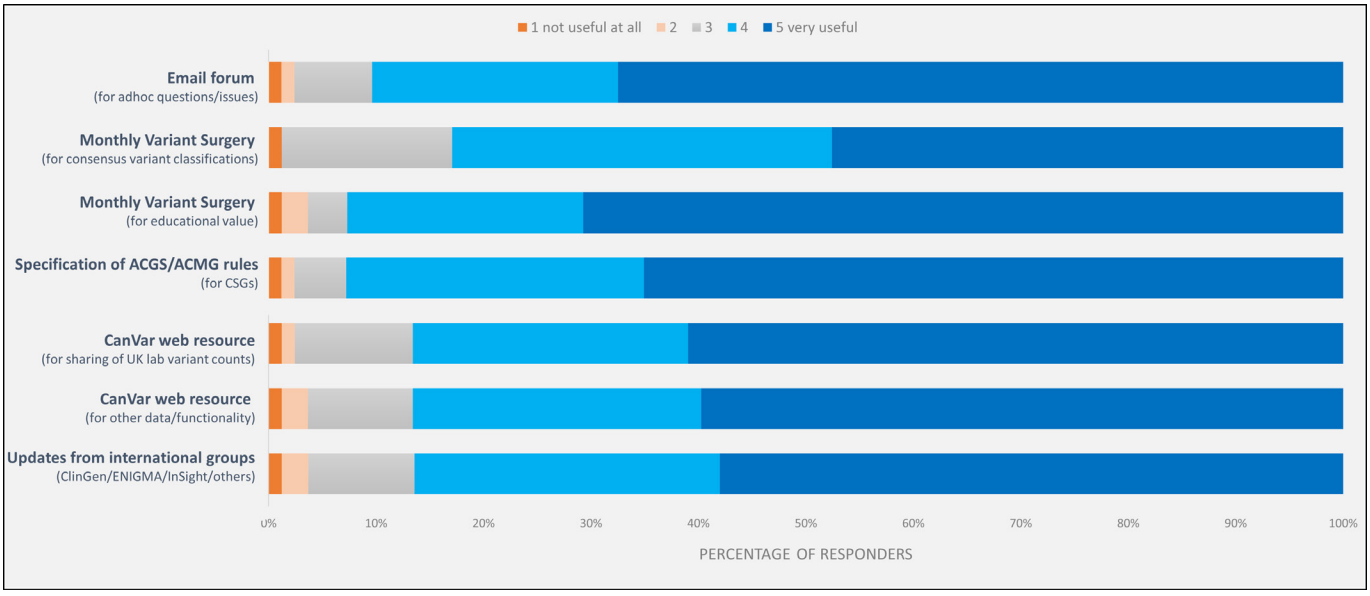

Figure 2 Perceived utility of CanVIG-UK activities regarding local practice in CSG variant interpretation of seven activities (5: very useful to 1: not useful; survey of CanVIG-UK members, performed on 29 October 2019, return rate 83/103 (81%)). ACGS, Association of Clinical Genomic Science; ACMG, American College of Medical Geneticists; CanVIG-UK, Cancer Variant Interpretation Group UK; CSG, cancer susceptibility gene.

example, provision to ENIGMA of the summary PHE UK laboratory data on BRCA1/BRCA2 variants.

- Fourth, CanVIG-UK consensus classifications (and underpinning evidence) are shared via ClinVar. CanVIG-UK is the first UK organisation to submit clinical-laboratory variant classifications to ClinVar.

Sustainability

Maintenance of a national multidisciplinary network, coordination of a regular teleconferenced MDMs and development of a data system is only feasible via sustained support. The activities of CanVIG-UK are currently supported by a Cancer Research UK Catalyst Award (CanGene-CanVar, @CangeneCanvar, C61296/A27223).

CONCLUSION

CanVIG-UK is a multidisciplinary group comprising >100 clinical scientists and senior genetics clinicians working in germline cancer genetics, with representation from across the 25 NHS molecular diagnostic laboratories of the UK and Ireland. Through CanVIG-UK, the UK clinical-laboratory germline cancer genetics community have evolved:

1. An email forum for real-time consultation on problematic variants.
2. A monthly teleconferenced MDM for detailed review of challenging variants and cases.
3. A national programme of using secure submissions of frequency data from PHE.
4. An online data system (CanVar-UK) for sharing variant-level data both publicly and within a secure community region.
5. Detailed, consensus UK guidance for the interpretation of variants in CSGs.
6. Fruitful interactions with international CSG variant interpretation endeavours.

In summary, we propose CanVIG-UK as an exemplar National Subspecialty Multidisciplinary Genomics Network. In this era of rapid emergence of genomic knowledge, such networks are becoming increasingly important to optimise collaborative specialist case review, information sharing and education.

Author affiliations
[1]Division of Genetics and Epidemiology, Institute of Cancer Research, Sutton, UK
[2]Wessex Regional Genetics Laboratory, Salisbury Hospital NHS Foundation Trust, Salisbury, UK
[3]Human Genetics and Genomic Medicin, Faculty of Medicine, University of Southampton, Southampton, UK
[4]Sheffield Diagnostic Genetics Service, Sheffield Children's NHS Foundation Trust, Sheffield, UK
[5]William Harvey Research Institute, Queen Mary University of London, London, UK
[6]Manchester Centre for Genomic Medicine and NW Laboratory Genetics Hub, Manchester University NHS Foundation Trust, Manchester, UK
[7]Yorkshire Regional Genetics Service, Leeds Teaching Hospitals NHS Trust, Leeds, UK
[8]Department of Clinical Genetics, Guy's and Saint Thomas' NHS Foundation Trust, London, UK
[9]Department of Clinical Genetics, Saint George's University Hospitals NHS Foundation Trust, London, UK
[10]Wessex Clinical Genetics Service, Princess Anne Hospital, Southampton, UK
[11]Oxford Molecular Genetics Laboratory, Churchill Hospital, Oxford, UK
[12]Institute of Medical Genetics, University Hospital of Wales, Cardiff, UK
[13]Faculty of Medicine, University of Southampton, Southampton, UK
[14]East Anglian Medical Genetics Service, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK
[15]Regional Genetics Service, Guy's and Saint Thomas' NHS Foundation Trust, London, UK
[16]Department of Molecular Genetics, Royal Devon and Exeter NHS Foundation Trust, Exeter, UK
[17]Division of Evolution and Genomic Sciences, School of Biological Sciences, Faculty of Biology Medicine and Health, The University of Manchester, Manchester, UK
[18]Department of Medical Genetics, National Institute for Health, Research Cambridge Biomedical Research Centre, University of Cambridge, Cambridge, UK
[19]Cancer Genetics Unit, Royal Marsden NHS Foundation Trust, London, UK

Twitter George J Burghel @BurghelG, Ian R Berry @LaughingGenome, Emma Woodward @ER_Woodward and Clare Turnbull @clare_turnbull

Acknowledgements We acknowledge the contribution of Jenny Lord and Andrew Douglas to discussions regarding splicing guidance.

Collaborators CanVIG-UK: Stephen Abbs, Patrick Tarpey, Jonathan Bruty, James Drummond, James Whitworth, Anne Ramsay Bowden, Marc Tischowitz, Eamonn Maher (Cambridge University Hospitals NHS Foundation Trust); Shirley Heggarty, Sean Hegarty, Rosalind Martin, Peter Logan, Claire Byrne (Belfast Health and Social

Care Trust); Yvonne Wallis, Samantha Butler, Rachel Hart, Lowri Hughes, Kim Reay, Kai-Ren Ong, Joanne Mason, Ian Tomlinson (Birmingham Women's and Children's NHS Foundation Trust); Ian Frayling, Sheila Palmer-Smith, Julian Sampson, Alex Murray (Cardiff and Vale University Health Board); Munaza Ahmed, Louise Kiely, Louise Busby, Claire Brooks, Alison Taylor-Beadling, Ajith Kumar (Great Ormond Street Hospital for Children NHS Foundation Trust); Vishakha Tripathi, Mina Ryten, Louise Izatt, Anjana Kulkarni, Adam Shaw, Joanna Campbell (Guy's and St Thomas' NHS Foundation Trust); Huw Thomas (St. Mark's Hospital, Northwick Park Hospital, Harrow); Daniel Chubb, Mary Alikian, Cankut Cubuk (Institute of Cancer Research); Rachel Robinson, Brendan Mullaney, Julian Adlard (Leeds Teaching Hospitals NHS Trust); Karen-Lynn Greenhalgh, Emma Howard (Liverpool Women's NHS Foundation Trust); Virginia Clowes, Angela Brady (London North West University Healthcare NHS Trust); George Burghel, Emma Woodward, Philip T Smith, Jade L Harris, Naomi L Bowers, Claire L Hartley, Ronnie Wright, Gareth Evans, Fiona Lalloo, Andrew Wallace (Manchester University NHS Foundation Trust); John Burn, James Tellez, Sarah Mackenzie, Helen Powell (Newcastle Upon Tyne Hospitals NHS Foundation Trust); Stephen Tennant, Joanna Tolmie, Dawn O'Sullivan (NHS Grampian, Aberdeen); Rosemarie Davidson, Jonathan Grant, Daniel Stobo, Aisha Ansari (NHS Greater Glasgow & Clyde); Jennie Murray, David Moore (NHS Lothian, Edinburgh); Rachael Tredwell, Joanne Field, Kirsty Bradshaw, Rachel Harrison (Nottingham University Hospital NHS Trust); Logan Walker (University of Otago, Christchurch, New Zealand); Trudi Mcdevitt, Marie Duff, Catherine Clabby (Our Lady's Children's Hospital, Crumlin, Dublin); Treena Cranston, Tina Bedenham, Evgenia Petrides, Lara Hawkes (Oxford University Hospitals NHS Foundation Trust); Fiona McRonald (Public Health England); Sian Ellard, Ruth Cleaver, Carole Brewer (Royal Devon And Exeter NHS Foundation Trust); Nick Woodwaer (Royal Free London NHS Foundation Trust); Stacey Daniels, Alison Callaway (Salisbury NHS Foundation Trust); Khalid Tobal, Shadi Albaba, Sarah Dell, Rodney Nyanhete, Richard Kirk, Mark Watson, Miranda Durkie, Jackie Cook, Hazel Clouston, Anne-Cecile Hogg (Sheffield Children's NHS Foundation Trust); Sabrina Talukdar, Lorraine Hawkes, Laura Cobbold, Kate Tatton-Brown, Helen Hanson, Katie Snape, Charlene Crosby, Ayaovi Hadonou Juan Carlos Del Rey Jimenez(St George's University Hospitals NHS Foundation Trust); Zoe Kemp, Terri Mcveigh, Clare Turnbull, Alice Garrett (The Royal Marsden NHS Foundation Trust); Cathal O'Brien (Trinity College Dublin, The University Of Dublin, Ireland); Laura Yarram, Kenneth Smith, Helen Williamson, Alan Donaldson (University Hospitals Bristol NHS Foundation Trust); Julian Barwell (University Hospitals of Leicester NHS Trust); Matilda Bradford (University Hospitals Plymouth NHS Trust); Lucy Side, Diana Eccles, Diana Baralle, Anneke Lucassen (University Hospital Southampton NHS Foundation Trust).

Contributors The manuscript was drafted by CT, AG, AC and MD. All other authors contributed to the final manuscript.

Funding This work is supported by the CRUK Catalyst Award CanGene-CanVar (C61296/A27223). ERW and DGE are supported by the Manchester National Institute for Health Research Biomedical Research Centre (IS-BRC-1215- 20007).

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

ORCID iDs
Alice Garrett http://orcid.org/0000-0001-8942-283X
George J Burghel http://orcid.org/0000-0001-9360-8194
Ian R Berry http://orcid.org/0000-0002-9710-4724
Diana M Eccles http://orcid.org/0000-0002-9935-3169
D Gareth Evans http://orcid.org/0000-0002-8482-5784
Clare Turnbull http://orcid.org/0000-0002-1734-5772

REFERENCES

1 Norbury G. Association of Clinical Genomic Science (ACGS) 2015-2016 Genetic Test Activity Audit2017.
2 Turnbull C, Sud A, Houlston RS. Cancer genetics, precision prevention and a call to action. *Nat Genet* 2018;50:1212–8.
3 Tandy-Connor S, Guiltinan J, Krempely K, LaDuca H, Reineke P, Gutierrez S, Gray P, Tippin Davis B. False-Positive results released by direct-to-consumer genetic tests highlight the importance of clinical confirmation testing for appropriate patient care. *Genet Med* 2018;20:1515–21.
4 Kilbride MK, Domchek SM, Bradbury AR. Ethical implications of direct-to-consumer hereditary cancer tests. *JAMA Oncol* 2018;4:1327–8.
5 . The Guardian, 2019. Available: https://www.theguardian.com/science/2019/jul/21/senior-doctors-call-for-crackdown-on-home-genetic-testing-kits
6 Eccles DM, Mitchell G, Monteiro ANA, Schmutzler R, Couch FJ, Spurdle AB, Gómez-García EB, ENIGMA Clinical Working Group. Brca1 and BRCA2 genetic testing-pitfalls and recommendations for managing variants of uncertain clinical significance. *Ann Oncol* 2015;26:2057–65.
7 Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, O'Donnell-Luria AH, Ware JS, Hill AJ, Cummings BB, Tukiainen T, Birnbaum DP, Kosmicki JA, Duncan LE, Estrada K, Zhao F, Zou J, Pierce-Hoffman E, Berghout J, Cooper DN, Deflaux N, DePristo M, Do R, Flannick J, Fromer M, Gauthier L, Goldstein J, Gupta N, Howrigan D, Kiezun A, Kurki MI, Moonshine AL, Natarajan P, Orozco L, Peloso GM, Poplin R, Rivas MA, Ruano-Rubio V, Rose SA, Ruderfer DM, Shakir K, Stenson PD, Stevens C, Thomas BP, Tiao G, Tusie-Luna MT, Weisburd B, Won H-H, Yu D, Altshuler DM, Ardissino D, Boehnke M, Danesh J, Donnelly S, Elosua R, Florez JC, Gabriel SB, Getz G, Glatt SJ, Hultman CM, Kathiresan S, Laakso M, McCarroll S, McCarthy MI, McGovern D, McPherson R, Neale BM, Palotie A, Purcell SM, Saleheen D, Scharf JM, Sklar P, Sullivan PF, Tuomilehto J, Tsuang MT, Watkins HC, Wilson JG, Daly MJ, MacArthur DG, Exome Aggregation Consortium. Analysis of protein-coding genetic variation in 60,706 humans. *Nature* 2016;536:285–91.
8 Pinard A, Salgado D, Desvignes J-P, Rai G, Hanna N, Arnaud P, Guien C, Martinez M, Faivre L, Jondeau G, Boileau C, Zaffran S, Béroud C, Collod-Béroud G. WES/WGS Reporting of Mutations from Cardiovascular "Actionable" Genes in Clinical Practice: A Key Role for UMD Knowledgebases in the Era of Big Databases. *Hum Mutat* 2016;37:1308–17.
9 Pinard A, Miltgen M, Blanchard A, Mathieu H, Desvignes J-P, Salgado D, Fabre A, Arnaud P, Barré L, Krahn M, Grandval P, Olschwang S, Zaffran S, Boileau C, Béroud C, Collod-Béroud G. Actionable genes, core databases, and locus-specific databases. *Hum Mutat* 2016;37:1299–307.
10 Szabo C, Masiello A, Ryan JF, Brody LC. The breast cancer information core: database design, structure, and scope. *Hum Mutat* 2000;16:123–31.
11 Fokkema IFAC, Taschner PEM, Schaafsma GCP, Celli J, Laros JFJ, den Dunnen JT. LOVD v.2.0: the next generation in gene variant databases. *Hum Mutat* 2011;32:557–63.
12 Greenblatt MS, Brody LC, Foulkes WD, Genuardi M, Hofstra RMW, Olivier M, Plon SE, Sijmons RH, Sinilnikova O, Spurdle AB, IARC Unclassified Genetic Variants Working Group. Locus-specific databases and recommendations to strengthen their contribution to the classification of variants in cancer susceptibility genes. *Hum Mutat* 2008;29:1273–81.
13 Easton DF, Deffenbaugh AM, Pruss D, Frye C, Wenstrup RJ, Allen-Brady K, Tavtigian SV, Monteiro ANA, Iversen ES, Couch FJ, Goldgar DE. A systematic genetic assessment of 1,433 sequence variants of unknown clinical significance in the BRCA1 and BRCA2 breast cancer-predisposition genes. *Am J Hum Genet* 2007;81:873–83.
14 Plon SE, Eccles DM, Easton D, Foulkes WD, Genuardi M, Greenblatt MS, Hogervorst FBL, Hoogerbrugge N, Spurdle AB, Tavtigian SV, IARC Unclassified Genetic Variants Working Group. Sequence variant classification and reporting: recommendations for improving the interpretation of cancer susceptibility genetic test results. *Hum Mutat* 2008;29:1282–91.
15 Spurdle AB, Healey S, Devereau A, Hogervorst FBL, Monteiro ANA, Nathanson KL, Radice P, Stoppa-Lyonnet D, Tavtigian S, Wappenschmidt B, Couch FJ, Goldgar DE, ENIGMA. ENIGMA-evidence-based network for the interpretation of germline mutant alleles: an international initiative to evaluate risk and clinical significance associated with sequence variation in BRCA1 and BRCA2 genes. *Hum Mutat* 2012;33:2–7.
16 Parsons MT, Tudini E, Li H, Hahnen E, Wappenschmidt B, Feliubadaló L, Aalfs CM, Agata S, Aittomäki K, Alducci E, Alonso-Cerezo MC, Arnold N, Auber B, Austin R, Azzollini J, Balmaña J, Barbieri E, Bartram CR, Blanco A, Blümcke B, Bonache S, Bonanni B, Borg Åke, Bortesi B, Brunet J, Bruzzone C, Bucksch K, Cagnoli G, Caldés T, Caliebe A, Caligo MA, Calvello M, Capone GL, Caputo SM, Carnevali I, Carrasco E, Caux-Moncoutier V, Cavalli P, Cini G, Clarke EM, Concolino P, Cops EJ, Cortesi L, Couch FJ, Darder E, de la Hoya M, Dean M, Debatin I, Del Valle J, Delnatte C, Derive N, Diez O, Ditsch N, Domchek SM, Dutrannoy V, Eccles DM, Ehrencrona H, Enders U, Evans DG, Farra C, Faust U, Felbor U, Feroce I, Fine M, Foulkes WD, Galvao HCR, Gambino G, Gehrig A, Gensini F, Gerdes A-M, Germani A, Giesecke J, Gismondi V, Gómez C, Gómez Garcia EB, González S, Grau E, Grill S, Gross E, Guerrieri-Gonzaga A, Guillaud-Bataille M, Gutiérrez-Enríquez S, Haaf T, Hackmann K, Hansen TVO, Harris M, Hauke J, Heinrich T, Hellebrand H, Herold KN, Honisch E, Horvath J, Houdayer C, Hübbel V, Iglesias S, Izquierdo A, James PA, Janssen LAM, Jeschke U, Kaulfuß S, Keupp K, Kiechle M, Kölbl A, Krieger S, Kruse TA, Kvist A, Lalloo F, Larsen M, Lattimore VL, Lautrup C, Ledig S, Leinert E, Lewis AL, Lim J, Loeffler M, López-Fernández A, Lucci-Cordisco E, Maass N, Manoukian S, Marabelli M, Matricardi L, Meindl A, Michelli RD, Moghadasi S, Moles-Fernández A, Montagna M, Montalban G, Monteiro AN, Montes E, Mori L, Moserle L, Müller CR, Mundhenke C, Naldi N, Nathanson KL, Navarro M, Nevanlinna H, Nichols CB, Niederacher D, Nielsen HR, Ong K-R, Pachter N, Palmero EI, Papi L, Pedersen IS, Peissel B, Perez-Segura P, Pfeifer K, Pineda M, Pohl-Rescigno E, Poplawski NK, Porfirio B, Quante AS, Ramser J, Reis RM, Revillion F, Rhiem K, Riboli B, Ritter J, Rivera D, Rofes P, Rump A, Salinas M, Sánchez de Abajo AM, Schmidt G,

Schoenwiese U, Seggewiß J, Solanes A, Steinemann D, Stiller M, Stoppa-Lyonnet D, Sullivan KJ, Susman R, Sutter C, Tavtigian SV, Teo SH, Teulé A, Thomassen M, Tibiletti MG, Tischkowitz M, Tognazzo S, Toland AE, Tornero E, Törngren T, Torres-Esquius S, Toss A, Trainer AH, Tucker KM, van Asperen CJ, van Mackelenbergh MT, Varesco L, Vargas-Parra G, Varon R, Vega A, Velasco Ángela, Vesper A-S, Viel A, Vreeswijk MPG, Wagner SA, Waha A, Walker LC, Walters RJ, Wang-Gohrke S, Weber BHF, Weichert W, Wieland K, Wiesmüller L, Witzel I, Wöckel A, Woodward ER, Zachariae S, Zampiga V, Zeder-Göß C, Lázaro C, De Nicolo A, Radice P, Engel C, Schmutzler RK, Goldgar DE, Spurdle AB, KConFab Investigators. Large scale multifactorial likelihood quantitative analysis of BRCA1 and BRCA2 variants: an enigma resource to support clinical variant classification. *Hum Mutat* 2019;40:1557–78.

17 Thompson BA, Spurdle AB, Plazzer J-P, Greenblatt MS, Akagi K, Al-Mulla F, Bapat B, Bernstein I, Capellá G, den Dunnen JT, du Sart D, Fabre A, Farrell MP, Farrington SM, Frayling IM, Frebourg T, Goldgar DE, Heinen CD, Holinski-Feder E, Kohonen-Corish M, Robinson KL, Leung SY, Martins A, Moller P, Morak M, Nystrom M, Peltomaki P, Pineda M, Qi M, Ramesar R, Rasmussen LJ, Royer-Pokora B, Scott RJ, Sijmons R, Tavtigian SV, Tops CM, Weber T, Wijnen J, Woods MO, Macrae F, Genuardi M. Application of a 5-tiered scheme for standardized classification of 2,360 unique mismatch repair gene variants in the insight locus-specific database. *Nat Genet* 2014;46:107–15.

18 Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL, ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of medical genetics and genomics and the association for molecular pathology. *Genet Med* 2015;17:405–24.

19 ClinGen variant curation expert panel. ClinGen TP53 expert panel specifications to the ACMG/AMP variant interpretation guidelines version 1, 2019. Available: https://clinicalgenome.org/site/assets/files/3876/clingen_tp53_acmg_specifications_v1.pdf2019

20 Brnich SE, Abou Tayoun AN, Couch FJ, Cutting GR, Greenblatt MS, Heinen CD, Kanavy DM, Luo X, McNulty SM, Starita LM, Tavtigian SV, Wright MW, Harrison SM, Biesecker LG, Berg JS, Clinical Genome Resource Sequence Variant Interpretation Working Group. Recommendations for application of the functional evidence PS3/BS3 criterion using the ACMG/AMP sequence variant interpretation framework. *Genome Med* 2019;12:3.

21 ClinGen variant curation expert panel. ClinGen CDH1 expert panel specifications to the ACMG/AMP variant interpretation guidelines version 2, 2019. Available: https://clinicalgenome.org/site/assets/files/3982/clingen_cdh1_acmg_specifications_v2.pdf

22 Mester JL, Ghosh R, Pesaran T, Huether R, Karam R, Hruska KS, Costa HA, Lachlan K, Ngeow J, Barnholtz-Sloan J, Sesock K, Hernandez F, Zhang L, Milko L, Plon SE, Hegde M, Eng C. Gene-Specific criteria for PTEN variant curation: recommendations from the ClinGen PTEN expert panel. *Hum Mutat* 2018;39:1581–92.

23 Tavtigian SV, Greenblatt MS, Harrison SM, Nussbaum RL, Prabhu SA, Boucher KM, Biesecker LG, ClinGen Sequence Variant Interpretation Working Group (ClinGen SVI). Modeling the ACMG/AMP variant classification guidelines as a Bayesian classification framework. *Genet Med* 2018;20:1054–60.

24 Ellard S, Baple EL, Berry I, Forrester N, Turnbull C, Owens M, Eccles DM, Abbs S, Scott R, Deans Z, Lester T, Campbell J, Newman W, McMullan D. ACGS best practice guidelines for variant classification in rare disease 2020: Association for Clinical Genomic Science (ACGS), 2020. Available: https://www.acgs.uk.com/quality/best-practice-guidelines/#VariantGuidelines

25 Luheshi L, Hall A, Baple E, Ellard S, McMullan D, Raza, S. Variant classication and interpretation workshop report, 2017. Assocation for clinical genomic science (ACGS) and PHG Foundation. Available: https://www.phgfoundation.org/documents/Variant%20classification%20and%20identification%20June%202017.pdf

26 Turnbull C, Scott RH, Thomas E, Jones L, Murugaesu N, Pretty FB, Halai D, Baple E, Craig C, Hamblin A, Henderson S, Patch C, O'Neill A, Devereau A, Smith K, Martin AR, Sosinsky A, McDonagh EM, Sultana R, Mueller M, Smedley D, Toms A, Dinh L, Fowler T, Bale M, Hubbard T, Rendon A, Hill S, Caulfield MJ, 100 000 Genomes Project. The 100 000 Genomes Project: bringing whole genome sequencing to the NHS. *BMJ* 2018;361:k1687.

27 NHS Genomic Medicine Service. NHS England, 2019. Available: https://www.england.nhs.uk/genomics/nhs-genomic-med-service/

28 Cox JH. Available: https://www.openpseudonymiser.org/About.aspx

Appendix 1: CanVIG-UK Consensus Specification for Cancer Susceptibility Genes of ACGS Best Practice Guidelines for Variant Classification (v1.2 03/03/20)

Guidance notes:

- For the following evidence items, CanVIG-UK have no additional specification for CSGs to add beyond that provided in ACGS Best Practice Guidelines for Variant Classification 2020[1]: PVS1, PS1, PS2, PM4, PM5, PM6, PP1, BS4, BP3, BP7 **(shaded white).**
- For the remaining evidence items, whilst remaining consistent with ACGS Best Practice Guidelines for Variant Classification 2020[1], there are more specific recommendations pertaining to CSGs contained within the CanVIG-UK Consensus Specification (**shaded grey).**
- A number of disease-specific expert panels have been established by the USA ClinGen Sequence Variant Interpretation (SVI) Working Group, generating disease/gene specific variant interpretation guidelines. Following evaluation within the CanVIG-UK group, in subsequent updates to the CanVIG-UK specification, we shall include specific recommendations regarding adoption and implementation of these disease/gene specific-guidance.

PVS1	_VSTR	_STR	_MOD	_SUP
Null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where LOF is a known mechanism of disease				
See LOF decision tree and criteria (Tayoun *et al* 2018)[2]				

PS1	_STR	_MOD	_SUP
Same amino acid change as a previously established pathogenic variant, regardless of nucleotide change			
Use at **Strong** for a missense variant under evaluation whereby there is a reference missense variant classified as (likely) pathogenic that results in the same amino acid change Use at **Moderate** for an initiation codon variant under evaluation whereby there is a reference variant in the initiation codon classified as (likely) pathogenic Use at **Supporting** for a donor/acceptor splice region variant under evaluation whereby there is a reference variant at the same base residue classified as (likely) pathogenic. The variant under evaluation must be predicted on *in silico* tools to be equally or more deleterious than the reference variant			

PS2, PM6	_ VSTR	_STR	_MOD	_SUP
***PS2**: De novo (both maternity and paternity confirmed) in a patient with the disease and no family history* ***PM6:** Assumed de novo, but without confirmation of paternity and maternity*				
See ClinGen Sequence Variant Interpretation Recommendation for *de novo* Criteria (PS2/PM6)[3] https://www.clinicalgenome.org/site/assets/files/3461/svi_proposal_for_de_novo_criteria_v1_0.pdf).				

PS3	_ VSTR	_STR	_MOD	_SUP
Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product				

For assays of protein function

	Discrimination	Controls	Reproducibility
Strong	relative protein activity assay or functional impact <25% compared to level for wildtype	≥10 'true positive' ≥10'true-negative'	≥2 laboratories OR results demonstrably reproducible from a single laboratory
Moderate		≥5 'true positive' ≥5 'true-negative'	
Supporting		≥2 'true positive' ≥2 'true-negative'	single laboratory

For assays of splicing function

Garrett A, *et al. J Med Genet* 2020;0:1–6. doi: 10.1136/jmedgenet-2019-106759

Very strong	**2 orthogonal assays:** exhibiting abnormal transcripts; no evidence of leakiness (see notes 4,8)
Strong	**1 assay:** exhibiting abnormal transcripts; no evidence of leakiness (see note 8)
Moderate	**≥1 assay:** exhibiting abnormal transcripts; evidence of leakiness (see note 8)
Supporting	**≥1 assay:** exhibiting abnormal/alternative transcripts which have been reported as present in normal controls (implying naturally occurring isoforms) (see note 12)
Do not apply	**≥1 assay:** exhibiting abnormal/alternative transcripts with evidence of extreme leakiness (see note 8)

Explanatory Notes (all functional assays):

- This criterion is for variant-specific analyses. Where functional data provides support at the gene rather than variant level (e.g. biochemical analysis), this should typically be incorporated within the phenotypic specificity criterion PP4
- To be adopted by CanVIG-UK, a published assay requires independent review by two CanVIG-UK registered clinical laboratory scientists
- Guidance for more quantitative evaluation of functional assays has recently been published by the Clinical Genome Resource Sequence Variant Interpretation Working Group (Brnich *et al* 2020)[4]. Evaluation of these approaches by CanVIG-UK is underway but additional training is required. We anticipate adoption as best practice of these more quantitative approaches for review of functional assays and will use the pan-CanVIG network to collate 'ratings' per assay

Explanatory Notes (assays of splicing function):

1. Experimental data may include quantitative assays (e.g. realtime-PCR, Sanger sequencing with formal quantitation of peak height, tape-station quantification of PCR products, minigene assay, RNAseq using NGS) and semi/non-quantitative assays (e.g. visual evaluation of the relative peak height of Sanger sequencing, gel-based evaluation and visualisation of reverse transcriptase PCR (RT-PCR) products, or analysis for evidence of nonsense mediated decay (e.g. where a SNV in trans with the putative splicing variant appears homozygous on RNA sequencing despite being heterozygous on DNA sequencing, indicating the loss of expression of the transcript containing the putative splicing variant))
2. Laboratory methodology should be appropriately validated: primers must have been tested in ≥5 independent normal control reactions, not necessarily run at the same time (i.e. primers could be validated using 5 normal controls across several runs or runs as a batch on a single run)
3. Assays must be performed in a diagnostically ISO accredited laboratory or recognized research laboratory with which direct consultation can be undertaken. **If evidence is derived from an alternative source (e.g. publication only), downgrade by one level of evidence.** All assays should evidence appropriate validations and controls (see note 2).
4. Combinations of assays deemed orthogonal include (a) two PCR-based assays using different primers (b) ≥2 different platforms e.g. RT-PCR and minigene
5. To attain very strong/strong, the criteria by which the disease mechanism is interpreted as loss of function should be met (as per PVS1 recommendations, Tayoun *et al* (2018)[2])
6. The exon in question must be present in the biologically relevant transcript
7. Splicing impact must fulfil one of the criteria below, **otherwise downgrade by one level of evidence**
    a) out of frame + predicted to undergo NMD + removal of >10% of the protein
    b) in-frame but removal of a critical hotspot (as listed in PM1)
    c) in-frame but removal of >10% of the protein
8. Although there will inevitably be gene by gene and exon by exon variation regarding the lower limit of % normal transcripts ('leakiness') at which normal protein function is maintained, this information is not always known. In the absence of specific data for a given gene/exon, the following thresholds of 'leakiness' should be applied:
    - **Evidence against leakiness**: ratio for allele of >80:20 (abnormal: normal) ==overall ratio of >40:60 (abnormal: normal)
    - **Evidence of some leakiness:** ratio for allele of >20:80 (abnormal: normal) ==overall ratio of >10:90 (abnormal: normal)
    - **Evidence of extreme leakiness:** ratio for allele of <20:80 (abnormal: normal) ==overall ratio of < 10:90 (abnormal: normal). Typically, abnormal transcript will be visible on gel but present only at extremely low level or not visible by Sanger sequencing

    The accuracy of different assays in correctly quantifying ratios of different transcripts will vary and is often poorly quantified. As improved data on the precision of different assays emerges, these

Garrett A, *et al. J Med Genet* 2020;0:1–6. doi: 10.1136/jmedgenet-2019-106759

standards will likely be amended
9. For ±1 or ±2, PVS1 criteria should be used instead of PS3
10. When PS3 is applied for splicing, PP3 (*in silico* evidence), PM4 (in-frame aberration) and PVS1 (truncating) cannot be applied
11. Although PP3 cannot be applied alongside PS3, the assay results for variants at the intron-exon boundaries should nevertheless be supported by *in silico* predictions (MaxEntScan ≥15% difference **OR** SSFL ≥5% difference), **otherwise downgrade by one level of evidence.** Exceptions where *in silico* concordance is not required: (i) U12 splice sites, (ii) TCCTTAAC at the 3' end, (iii) variants outside of intron-exon boundaries (namely 5': Last 3 bases of exon plus 8 bases on intron 3':12 bases of intron plus 2 bases of exon)
12. Naturally occurring (i.e. non-pathogenic) splice variants have been catalogued by expert groups (for example ENIGMA at https://enigmaconsortium.org/library/general-documents/enigma-classification-criteria/)[5] and in reference transcriptome resources (for example GTEX, https://gtexportal.org/home/)[6]

PS4 _VSTR _STR _MOD _SUP

The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls

Vstrong	$P_{exact} \leq 0.0025$
Strong	$P_{exact} \leq 0.05$
Mod	$P_{exact} \leq 0.1$
Sup	$P_{exact} \leq 0.2$

	Cases	Controls	Total
Variant	a	b	a+b
WT	c	d	c+d
Total	a+c	b+d	a+b+c+d

Explanatory Notes:
- The P_{exact} is generated from Fishers exact 2-way case control comparison (ad/bc)
- Non-duplicated, robustly genotyped case data and control data from equivalent ethic groups are required
- For Western European case data, comparison to the gnomAD NFE population is recommended (i.e. 64,603 NFE individuals for gnomAD v2.1)
- Where there is no count for the variant in gnomAD v2.1, it is not currently possible to ascertain the correct frequency for the wildtype allele. It is recommended that frequency is inferred from inspection at a nearby base at which a variant has been called. If this is not possible, we recommend using a denominator of 90% of the population size (ie 90% x 64,603 NFE individuals, ie 58,143 individuals) to approximate for the frequency at that base accounting for failed calls
- The p-value does not reflect effect size. Therefore, the Odds Ratio (OR) from this case control comparison (ad/bc) should be consistent with the effect size anticipated for that gene type
  - For a 'high penetrance' gene or variant, OR >4 for unselected cancer series or OR>8 for enriched familial cases
  - For an 'intermediate penetrance' gene or reduced penetrance variant in high penetrance gene, OR >2 for unselected cancer series or OR>4 for enriched familial cases
- If the control frequency is 0, the Haldane-Anscombe correction is required to generate an OR (add 0.5 to cells a,b,c,d)
- If there is uncertainty regarding duplicates in the case series, a commensurately more stringent p-value should be applied
- For non-coding variants, restriction to the WGS partition of gnomAD is required
- Where paired numerator-denominator frequencies are unavailable, a case-counting approach is required, which takes into account the specificity of phenotype observed in the proband +/- family. This approach does not take into account the denominator of the reference case series. For *TP53* and *PTEN*, case-counting guidance has been issued via the respective ClinGen expert groups[7] [8]. For MMR, a case-counting approach is under development by CanVIG-UK
- For other rare syndrome cancer susceptibility genes, the UK-ACGS rare disease guidance can be applied. Namely: PS4 can be used at a moderate level of evidence if the variant has been previously identified in multiple (two or more) unrelated affected probands/families with a pathognomonic spectrum of disease, or at a supporting level of evidence if previously identified

in one unrelated affected individual, and has not been reported in gnomAD (in a matched ethnic group)

PM1, PP2 _MOD _SUP
PM1: *Located in a mutational hot spot and/or critical and well-established functional domain (e.g. active site of an enzyme) without benign variation* ***PP2:*** *Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease*
Use PM1 for missense variants arising in a CSG domain well characterised as a "hotspot" for pathogenic missense variants Use PM1 at **Moderate** for a variant in a mutational hotspot at which there is no benign variation Use PM1 at **Supporting** for a variant in a mutational hotspot at which there is some benign variation Use PP2 at **Supporting** where there is overall constraint for missense variation at the level of the region/exon/gene (Z≥3.09) **Explanatory Notes:** • The majority of CSGs act by loss of function. Hence, for many of these genes, the majority of established pathogenic variants are truncating (early linkage analyses, agnostic to mechanism, support this). Examples: *BRCA1, BRCA2, PALB2, RAD51C, RAD51D, MLH1, MSH2, MSH6, PMS2.* However, in these genes, there are typically specific domains in which missense variation at key residues can cause loss of function. Where benign variation typically also occur in these regions, PM1_sup can be used. e.g. residues listed in by ENIGMA within *BRCA1* (BRCT and RING domains) and *BRCA2* (DNA binding domain) (https://enigmaconsortium.org/wp-content/uploads/2018/10/ENIGMA_Rules_2017-06-29-v2.5.1.pdf) [5]

PM2 _MOD _SUP
Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium

Use at **Moderate:** where 0 observations of the variant in control series >50,000 individuals
Use at **Supporting** where 1 observation on the variant in control series >50,000 individuals
Explanatory Notes:
• Ensure that sequencing coverage is sufficient
• Be cautious in using this criterion for small insertions/deletions, as sequencing approaches/analytical methodologies can result in wide variation in calling of these variant types in NGS/exome/genome data
• If PS4 has been used for case control data
  ○ The same dataset cannot be re-used for PM2
  ○ An alternative dataset may be used for PM2 of >50,000 individuals of the same ethnicity as that applied for PS4 **OR**
  ○ An alternative dataset may be used for PM2 of >50,000 individuals of ethnicity(ies) different to those for PS4, e.g. If the gnomAD NFE has been used for PS4, the remainder of the GNOMAD populations may be used for PM2 (eg 76,853 individuals non NFE from gnomAD v2.1, if NFE used for PS4)
• If PS4 case control data has NOT been used
  ○ A dataset >50,000 individuals of the same ethnicity as your reference case/family **must** be used for PM2

PM3 _STR _MOD _SUP
For recessive disorders, detected in trans with a pathogenic variant
Use where variant found in trans with a pathogenic variant and the patient-level clinical features match those anticipated for the gene in question Use at **Strong** where variant found in ≥2 unrelated cases, and the features are distinctive for that gene Use at **Moderate** where variant found in 1 case, and the features are distinctive for that gene Use at **Supporting** where variant found in 1 case, and the features are distinctive for a set of genes **Explanatory Notes:**

Garrett A, *et al. J Med Genet* 2020;0:1–6. doi: 10.1136/jmedgenet-2019-106759

- Comprehensive analysis should be undertaken for the gene to exclude an alternative second pathogenic variant (e.g. including MLPA) in that gene
- Comprehensive analysis should be undertaken for all other genes for which the phenotypic features overlap
- Requires testing of parents (or offspring) to confirm phase
- Can use for homozygous variants but downgrade by one evidence level (as per ClinGen SVI points-based system)[9]

PM4 _MOD _SUP

Protein length changes as a result of in-frame deletions/insertions in a non-repeat region or stop-loss variants

Use at **Moderate** for
- In-frame insertions/deletions for which PVS1 is not applicable (Tayoun et al 2018[2])

Use at **Supporting** for
- In-frame insertions/deletions of a single amino-acid

PM5 _MOD _SUP

Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before

Use at **Moderate** if
- Reference variant is classified as pathogenic [OR likely pathogenic and reported in >1 individual]

Use at **Supporting** if
- Reference variant is classified likely pathogenic and only reported in 1 individual

PM6: see above (PS2)

PP1 _STR _MOD _SUP

Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease

- See Jarvik and Browning (2016)[10]

PP2: see above (PM1)

PP3 _SUP

Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact)

- Protein impact: Using a ***predefined*** strategy of
  - 3/3 tools (one tool may be marginally below threshold)
    - SIFT (deleterious), Polyphen HumVar ≥ (probably damaging) plus:
      - Align GVGD (C45, C65), (for *BRCA1, BRCA2*) **OR**
      - MAPP (bad) (for MMR genes) **OR**
      - CADD (>15) (for any other CSG)
  - Or use Revel (>0.7) as a single score
- Splicing impact:
  - Intron-exon boundary: MaxEnt >15% difference **AND** SSFL >5% difference[11]
  - Deep intronic: predicted creation of a novel splice site of any strength, absent in the normal sequence

PP4 _STR _MOD _SUP

Patient's phenotype or family history is highly specific for a disease with a single genetic aetiology

Level	Points	Cellular/molecular phenotype	Example
-	0.5	Moderately predictive for germline aberration of one of a small set of genes	MSI (for mismatch repair deficiency)
Sup	1	Highly predictive for germline aberration of one of a small set of genes	Aberration on mitomycin-induced chromosomal breakage (for genes related to Fanconi Anaemia)
Sup	1	Moderately predictive for germline aberration of the specific gene	LOH at chromosomal locus of tumour-suppressor gene
			Loss on immunohistochemistry of single protein eg MSH6, PMS2
Mod	2	Highly predictive for germline aberration of the specific gene	Depletion of BRCA2 in lymphocytes **and** aberration on mitomycin-induced chromosomal breakage (for *BRCA2*-related Fanconi Anaemia)
			Loss on immunohistochemistry of paired mismatch repair proteins e.g. *MSH2* **and** *MSH6*
			Loss of *MLH1+PMS2* on immunohistochemistry **and** normal *MLH1* promoter methylation (for *MLH1*-related mismatch repair deficiency)

Explanatory Notes:

- For CSGs, PP4 is largely applied for a cellular/molecular phenotype that implicates a particular gene or gene-set
- Comprehensive analysis of the gene and related genes should have been undertaken to exclude an alternative pathogenic variant (including MLPA)
- Individuals/tumours included must have been demonstrated to carry the germline variant
- Evidence can be summed across multiple cases:
  - Total points: Suporting:1; Moderate: 2; Strong: 4
  - Only one individual per family can contribute
- Up to two ***independent*** tumour phenotype assays can be included per case (e.g. MSI AND LOH). Strongly correlated tumour phenotypes from the same case cannot both be included, e.g. MSI and IHC
- For CSGs the high-level clinical phenotype is often too non-specific (e.g. breast and/or ovarian cancer). For a number of pleiomorphic rare tumour and/or syndromic presentations of cancer susceptibility, the specificity of high level clinical phenotype has been captured within PS4 within the case counting guidance (e.g. ClinGen criteria for *CDH1, PTEN, TP53*[7 8 12]). For other pleiomorphic rare tumour and/or syndromic presentations (e.g. MEN1, HLRCC), such specifications are awaited. If molecular data have been used within PS4 as part of a case counting exercise, then PP4 should not be applied

PP5 _SUP

Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation

- Any classification of LP/P after 2016 from
  - ≥2 accredited North American diagnostic laboratories OR
  - a single North American diagnostic laboratory where the utilised evidence is clearly cited
  - an approved ClinGen Expert Group (3 star on ClinVar), e.g. INSIGHT, ENIGMA
- When a single laboratory has classified as LP/P with provision of insufficient detail, it is advised that the individual laboratory is contacted to procure directly the evidence used for classification

Garrett A, *et al. J Med Genet* 2020;0:1–6. doi: 10.1136/jmedgenet-2019-106759

Additional comments:

- This is an **exceptional** application, as per UK-ACGS specification. For widely tested cancer susceptibility genes, classifications by large laboratories may have been derived from their substantial series of case data not otherwise publicly available

BA1/BS1 _SA _STR

Allele frequency is "too high" for disorder (ExAC or GnomAD)

Use **BA1** as **Stand_Alone** when allele frequency in a large dataset of heterogeneous outbred population (>10,000 individuals) is: >1% or >0.5% (*BRCA1, BRCA2, MLH1, MSH2*)
Use **BS1** as **Strong** when allele frequency in a heterogeneous outbred population is > value specified for specific gene by respective expert group

Explanatory Notes:

- Occasional pathogenic founder mutations occurring at an appreciable frequency in Western Europeans were identified in early characterisation of autosomal dominant CSGs (e.g. *CHEK2* 1100delC at ~0.6%). However, large volumes of sequencing in Western Europeans have now been performed for routinely tested CSGs. The reduction of the threshold of BA1 from 5% to 1% for CSGs and to 0.5% for very well characterised CGSs is predicated on existence of sufficiently high volumes of sequencing data to preclude the existence of hitherto undescribed common founder mutations

BS2/BP2 _STR _SUP

BS2: *Observation in controls inconsistent with disease penetrance. Observed in a healthy adult individual for a* ***recessive (homozygous),*** *dominant (heterozygous), or X-linked (hemizygous) disorder, with full penetrance expected at an early age*
BP2: *Observed* ***in trans*** *with a pathogenic variant for a fully penetrant dominant gene/disorder or observed in cis with a pathogenic variant in any inheritance pattern*

Use BP2 or BS2 at **Supporting** where no further genotyping or clinical/cellular phenotyping is possible
Use BS2 at **Strong** where

- laboratory analysis has been repeated using an orthogonal approach (e.g. different primers) to confirm homozygosity for allele AND
- patient is of age at which biallelic pathogenic variants would be anticipated to be penetrant for a distinctive phenotype AND
- patient has been actively examined to exclude relevant phenotype AND/OR had analysis of cellular phenotype

OR the homozygote is observed in a specified control population in addition to a heterozygote frequency meeting BS1
Use BP2 at **Strong** where

- alleles have been confirmed as in trans AND
- patient is of age at which biallelic pathogenic variants would be anticipated to be penetrant for a distinctive phenotype AND
- patient has been actively examined to exclude relevant phenotype AND/OR had analysis of cellular phenotype

Explanatory Notes:

- BS2 should only be used in the recessive context and for observation of a **homozygote**
- BP2 is used for where the variant is reported as a **compound heterozygote** in conjunction with a pathogenic variant in unaffected individual
- For cancer susceptibility genes, **BP2 and BS2** should only be used for those genes in which typical (non-hypomorphic) biallelic variants cause a recognised phenotype that is fully penetrant from infancy. Such genes include *BRCA2, PALB2, MLH1, MSH2, MSH6, PMS2*

Garrett A, *et al. J Med Genet* 2020;0:1–6. doi: 10.1136/jmedgenet-2019-106759

BS3 _STR _SUP

Well-established in vitro or in vivo functional studies show no damaging effect on protein function or splicing

For assays of protein function

	Discrimination	Controls	Reproducibility
Strong	relative protein activity assay or functional impact	≥10 'true positive' ≥10 'true-negative'	≥2 laboratories OR Results demonstrated as reproducible in single laboratory
Sup	>25% compared to level for wildtype	≥2 'true positive' ≥2 'true-negative'	single laboratory

For assays of splicing function

Strong	**1 assay:** with no evidence of abnormal transcripts (% normal transcript>90%)	ISO accredited laboratory or recognized research laboratory with which direct consultation can be undertaken
Sup	**1 assay:** with no evidence of abnormal transcripts (% normal transcript>90%)	alternative source of evidence (e.g. publication)

Explanatory Notes:

- BS3 should only be applied for an assay of protein function whereby the assay has been validated for variants in the relevant domain to ensure that the mechanism of pathogenicity captured by the assay in question is relevant to that variant
- BS3 should not be applied for an assay of protein function when *in silico* tools predict effect on splicing and/or for the first or last three bases of the exon
- A splicing assay can only be used for BS3 for intronic variants and those in the first or last three bases of the exon
- Evidence of amplification of both alleles is required (i.e. sequencing should demonstrate the SNV in question or another nearby SNV, on the background of the wildtype sequence). This is necessary to exclude generation of a 'normal' RNA result when the splicing aberration has not been detected by the assay used (e.g. due to intron retention, size too large for the PCR to amplify)
- When BS3 is applied for splicing, BP4 (*in silico* evidence), **cannot** be applied. For assays of protein function BS3 and BP4 can be combined.
- For specification of acceptable assays and QC standards, see PS3

BS4 _STR

Non segregation with disease

- See Jarvik and Browning (2016)[10]
- For cancer susceptibility genes for which the phenotype is non-specific and/or feature age-related/reduced penetrance, phenocopies or hypomorphic variants, expert review is recommended for application of BS4 pertaining to non-segregation.

BP1 _SUP

Missense variant in a gene for which primarily truncating variants are known to cause disease

Use at **Supporting** for genes/gene regions in which >95% of reported pathogenic variants are truncating

Explanatory note:

- Can be used outside of BRCT and RING domains for *BRCA1* and outside of DNA-binding domain for *BRCA2.* Other examples of genes for which criterion can be used include *PALB2* and *APC*
- Variant should be evaluated to exclude splicing impact
- Should not be used if the specific amino acid residue is highly conserved in mammals

BP2: see above (BS2)

Garrett A, *et al. J Med Genet* 2020;0:1–6. doi: 10.1136/jmedgenet-2019-106759

BP3 _SUP

In-frame deletions in a repetitive region without a known function

- Particularly relevant to poorly conserved regions

BP4 _SUP

Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc.)

- Splicing impact:
  - Intron-exon boundary: Minimal difference in readings for each of MaxEnt AND SSFL
  - AND no evidence of prediction of exonic/deep intronic novel splice site of any strength

AND

- Protein impact: Using a ***predefined*** strategy of
  - 3/3 tools (one tool may be marginally above threshold)
    - SIFT (tolerated), Polyphen HumVar (benign) plus:
      - Align GVGD (C0, C15), (for BRCA1, BRCA2)
      - MAPP (good) (for MMR genes)
      - CADD (<10) (for any other CSG)
  - Or Revel (<0.4) as a single score

BP5 _SUP

Variant found in a case with an alternate molecular basis for disease

This should not be applied for autosomal dominant incompletely penetrant non-syndromic genes associated with common cancers e.g. HBOC (hereditary breast and ovarian cancer)

Explanatory note:

- Co-occurrence of ≥2 pathogenic variants in different cancer susceptibility genes is widely reported. Typically, the phenotype exhibited is indistinguishable from that of a single pathogenic variant

BP6 _SUP

Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation

Explanatory Notes:

- Any classification of LB/B after 2016 from
  - ≥2 accredited North American diagnostic laboratory OR
  - a single North American diagnostic laboratory where the utilised evidence is clearly listed
  - ClinGen Expert Group, e.g. INSIGHT, ENIGMA
- When a single laboratory has classified as LB/B with provision of insufficient detail, it is advised that the individual laboratory is contacted to procure directly the evidence used for classification

Additional comments:

This is an **exceptional** application, as per UK-ACGS specification, as for commonly tested cancer susceptibility genes, classifications by large laboratories may have be derived from their substantial series of case data not otherwise publicly available

BP7 _SUP

A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved

- Not to be used if any cause for suspicion of an impact on splicing

References

1. Ellard S, Baple EL, Berry I, Forrester N, Turnbull C, Owens M, Eccles DM, Abbs S, Scott R, Deans Z, Lester T, Campbell J, Newman W, McMullan D. ACGS Best Practice Guidelines for Variant Classification 2020: Association for Clinical Genetics Science (ACGS), 2020.
2. Abou Tayoun AN, Pesaran T, DiStefano MT, Oza A, Rehm HL, Biesecker LG, Harrison SM. Recommendations for interpreting the loss of function PVS1 ACMG/AMP variant criterion. *Human mutation* 2018;39(11):1517-24. doi: 10.1002/humu.23626 [published Online First: 2018/09/08]
3. ClinGen. ClinGen Sequence Variant Interpretation Recommendation for de novo Criteria (PS2/PM6) version 1.0-, 2018.
4. Brnich SE, Abou Tayoun AN, Couch FJ, Cutting GR, Greenblatt MS, Heinen CD, Kanavy DM, Luo X, McNulty SM, Starita LM, Tavtigian SV, Wright MW, Harrison SM, Biesecker LG, Berg JS. Recommendations for application of the functional evidence PS3/BS3 criterion using the ACMG/AMP sequence variant interpretation framework. *Genome medicine* 2019;12(1):3. doi: 10.1186/s13073-019-0690-2 [published Online First: 2020/01/02]
5. Spurdle A. ENIGMA BRCA1/2 Gene Variant Classification Criteria 2017 [Available from: https://enigmaconsortium.org/wp-content/uploads/2018/10/ENIGMA_Rules_2017-06-29-v2.5.1.pdf.
6. The Genotype-Tissue Expression (GTEx) project. *Nature genetics* 2013;45(6):580-5. doi: 10.1038/ng.2653 [published Online First: 2013/05/30]
7. Mester JL, Ghosh R, Pesaran T, Huether R, Karam R, Hruska KS, Costa HA, Lachlan K, Ngeow J, Barnholtz-Sloan J, Sesock K, Hernandez F, Zhang L, Milko L, Plon SE, Hegde M, Eng C. Gene-specific criteria for PTEN variant curation: Recommendations from the ClinGen PTEN Expert Panel. *Human mutation* 2018;39(11):1581-92. doi: 10.1002/humu.23636 [published Online First: 2018/10/13]
8. Savage SA. TP53 Rule Specifications for the ACMG/AMP Variant Curation Guidelines clinicalgenome.org: ClinGen; 2019 [Available from: https://clinicalgenome.org/site/assets/files/3876/clingen_tp53_acmg_specifications_v1.pdf2019.
9. ClinGen. SVI Recommendation for in trans Criterion (PM3) - Version 1.0, 2019.
10. Jarvik GP, Browning BL. Consideration of Cosegregation in the Pathogenicity Classification of Genomic Variants. *American journal of human genetics* 2016;98(6):1077-81. doi: 10.1016/j.ajhg.2016.04.003 [published Online First: 2016/05/31]
11. Houdayer C, Caux-Moncoutier V, Krieger S, Barrois M, Bonnet F, Bourdon V, Bronner M, Buisson M, Coulet F, Gaildrat P, Lefol C, Leone M, Mazoyer S, Muller D, Remenieras A, Revillion F, Rouleau E, Sokolowska J, Vert JP, Lidereau R, Soubrier F, Sobol H, Sevenet N, Bressac-de Paillerets B, Hardouin A, Tosi M, Sinilnikova OM, Stoppa-Lyonnet D. Guidelines for splicing analysis in molecular diagnosis derived from a set of 327 combined in silico/in vitro studies on BRCA1 and BRCA2 variants. *Human mutation* 2012;33(8):1228-38. doi: 10.1002/humu.22101 [published Online First: 2012/04/17]
12. ClinGen CDH1 Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 2, 2019.

Appendix 2: Example of CanVIG-UK classification summary report

Variant classification by Can-VIG UK (Cancer Variant Interpretation UK) Can-VIG UK is a working group convened on behalf of the UK ACGS (Association of Clinical Genetic Scientists), which includes registered clinical scientists and clinical geneticists representing the following UK Regional molecular diagnostic laboratories: Aberdeen, Belfast, Birmingham, Bristol, Cambridge, Cardiff, Dublin, Exeter, Glasgow, GOSH, Guy's, Leeds, Liverpool (Cheshire & Merseyside), Oxford, Manchester, Newcastle, Nottingham, Sheffield , SW Thames (St George's), Wessex (Salisbury/Southampton), University Hospitals of Leicester.					
Submitter	Dr Clare Turnbull MD PhD FRCP FRCPath			**Date**	15/11/19
Gene	BRCA1	**Transcript**	NM_007294.3 ENST00000357654 LRG_292t1	**Variant**	c.53T>C (p.Met18Thr)
Population data	The variant was observed in 7 independent UK families undergoing clinical diagnostic testing, the denominator of which dataset of clinical testing was 25,773. Case control comparison against ethnically matched population data (7/25,773 in familial cases against 0/64,603 GNOMAD NFE controls p_{exact}= 0.0015 There are additional reports of this variant in ClinVar (6), BIC (3) LOVD3 (22), UMD(7), DMuDB(7) The variant is absent in the remainder of the GNOMAD populations (76,853 individuals)				
Prediction (based on variant type/location), IN silico tools	**AlignGVGD class:** C45 **SIFT prediction:** deleterious **MAPP prediction:** bad **Polyphen2 HumVar prediction:** benign **CADD scaled score [0-99]:** 16.18 **SuSPect score [0-100]:** 95				
Functional data	Findlay *et al.* 2018: Non-functional via saturation editing analysis using haploid *BRCA1* construct				
Segregation data					
De novo data					
Allelic data (biallelic observations)					
Other classifications	Ambry LP 2018, Gene Dx LP 2018, Counsyl LP 2018 Color LP 2018 Enigma 2019 (multifactorial analysis): pathogenic				
Other					

PATHOGENIC Criteria	**Weight (supporting, moderate, strong, very strong)**	**BENIGN Criteria**	**Weight (supporting, strong)**
PVS1(null)		BS1/BA1 (controls)	
PS4 (case control)	Very strong	BP4 (in silico)	
PM2 (absent control)	Mod	BP1 (only trunc)	
PP3 (in silico)		BP7 (synonymous)	
PM5 (same residue)		BP3 (in frame, no func)	
PM1 (hot spot)		BS3 (functional assay)	
PP1 (Segregation)		BS4 (non segregation)	
PS3 (functional assay)	Strong	BP2 (biallelic)	
PM3 (biallelic)		BP6 (other databases)	
PP5 (other databases)	Sup	Alternative cause (BP5)	
Specific phenotype (PP4)			
De novo (PM6, PS2)			
Total	1 very strong, 1 strong, 1 mod, 1 sup	**Total**	
Classification	5-Pathogenic		

Appendix 3: CanVIG-UK Consensus Guidance for Variants of Reduced Penetrance in High Penetrance Cancer Susceptibility Genes

- Variant interpretation and classification should be undertaken using the ACMG framework (with ACGS and CanVIG-UK specifications)
- If any of the below criteria are met, the variant should be assigned the relevant ACMG class but with addendum of "reduced penetrance"
- The report should reference and recommend the nationally ratified clinical management recommendations for that gene for variants of reduced penetrance
- Clinical management recommendations for variants of reduced penetrance for each gene should be established by disease-specific experts

Criterion 1: Down-modification of classic biallelic phenotype

Abnormal physical AND cellular phenotype associated with biallelic mutations is present but notably milder

Example: BRCA2-related Fanconi anaemia:

- Cancer is not penetrant by 5 years **AND**
- Congenital abnormalities and physical features are mild **AND**
- Incomplete functional abrogation of chromosomal breakage following mitomycin C exposure **OR** BRCA2-specific assays show only modest depletion of BRCA2 in quantity and/or function

Criterion 2: Well calibrated assay gives intermediate effect

- Highly predictive and well-calibrated published functional assay demonstrate intermediate effect, ie significant impairment of protein function but not at level demonstrated for truncating mutations in gene (e.g. Guidugli et al for BRCA2[13 14], Findlay et al 2019 for BRCA1[15])

Criterion 3: Segregation analysis gives lower estimate of penetrance

- Formal genetic epidemiologic analyses demonstrate variant to be associated with disease but of penetrance statistically significantly reduced compared to established estimates eg: BRCA1 c.5096G>A p.Arg1699Gln[16 17]

12. ClinGen CDH1 Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 2, 2019.
13. Guidugli L, Carreira A, Caputo SM, Ehlen A, Galli A, Monteiro AN, Neuhausen SL, Hansen TV, Couch FJ, Vreeswijk MP. Functional assays for analysis of variants of uncertain significance in BRCA2. *Human mutation* 2014;35(2):151-64. doi: 10.1002/humu.22478 [published Online First: 2013/12/11]
14. Guidugli L, Shimelis H, Masica DL, Pankratz VS, Lipton GB, Singh N, Hu C, Monteiro ANA, Lindor NM, Goldgar DE, Karchin R, Iversen ES, Couch FJ. Assessment of the Clinical Relevance of BRCA2 Missense Variants by Functional and Computational Approaches. *American journal of human genetics* 2018;102(2):233-48. doi: 10.1016/j.ajhg.2017.12.013 [published Online First: 2018/02/06]
15. Findlay GM, Daza RM, Martin B, Zhang MD, Leith AP, Gasperini M, Janizek JD, Huang X, Starita LM, Shendure J. Accurate functional classification of thousands of BRCA1 variants with saturation genome editing. *bioRxiv* 2018
16. Spurdle AB, Whiley PJ, Thompson B, Feng B, Healey S, Brown MA, Pettigrew C, Van Asperen CJ, Ausems MG, Kattentidt-Mouravieva AA, van den Ouweland AM, Lindblom A, Pigg MH, Schmutzler RK, Engel C, Meindl A, Caputo S, Sinilnikova OM, Lidereau R, Couch FJ, Guidugli L, Hansen T, Thomassen M, Eccles DM, Tucker K, Benitez J, Domchek SM, Toland AE, Van Rensburg EJ, Wappenschmidt B, Borg A, Vreeswijk MP, Goldgar DE. BRCA1 R1699Q variant displaying ambiguous functional

abrogation confers intermediate breast and ovarian cancer risk. *Journal of medical genetics* 2012;49(8):525-32. doi: 10.1136/jmedgenet-2012-101037 [published Online First: 2012/08/15]

17. Moghadasi S, Meeks HD, Vreeswijk MP, Janssen LA, Borg A, Ehrencrona H, Paulsson-Karlsson Y, Wappenschmidt B, Engel C, Gehrig A, Arnold N, Hansen TVO, Thomassen M, Jensen UB, Kruse TA, Ejlertsen B, Gerdes AM, Pedersen IS, Caputo SM, Couch F, Hallberg EJ, van den Ouweland AM, Collee MJ, Teugels E, Adank MA, van der Luijt RB, Mensenkamp AR, Oosterwijk JC, Blok MJ, Janin N, Claes KB, Tucker K, Viassolo V, Toland AE, Eccles DE, Devilee P, Van Asperen CJ, Spurdle AB, Goldgar DE, Garcia EG. The BRCA1 c. 5096G>A p.Arg1699Gln (R1699Q) intermediate risk variant: breast and ovarian cancer risk estimation and recommendations for clinical management from the ENIGMA consortium. *Journal of medical genetics* 2018;55(1):15-20. doi: 10.1136/jmedgenet-2017-104560 [published Online First: 2017/05/12]

Supplementary table 1: Variants reclassified to date in CanVIG-UK multidisciplinary meeting; RP: reduced penetrance

Date of CanVIG-UK review	Gene	Variant	Previous UK clinical classifications: most common (others)	CanVIG-UK consensus classification	Submission to ClinVar
Nov-17	BRCA1	c.53T>C p.Met18Thr	4 (3,5)	5	Y
Dec-17	BRCA2	c.8063T>C p.Leu2688Pro	3 (4,5)	5	Y
Jan-18	BRCA1	c.4096+3A>G	3 (2, 4)	2	Y
Feb-18	BRCA1	c.442-22_442-13del	3 (4,5)	4	Y
Mar-18	MLH1	c.122A>G p.Asp41Gly	3 (4,5)	5	Y
April-18	TP53	c.998G>A p.Arg333His	3 (4)	3	Y
Jun-18	BRCA2	c.9302T>G p.Leu3101Arg	4 (3,5)	5	Y
Jul-18	BRCA1	c.4963T>C p.Ser1655Pro	4 (3,5)	5	Y
Jul-18	BRCA1	c.4964C>T p.Ser1655Phe	4 (3,5)	5	Y
Jul-18	BRCA1	c.5207T>C p.Val1736Ala	3 (4)	5	Y
Aug-18	BRCA1	c.4357+6T>C	3 (4,5)	5	Y
Aug-18	BRCA2	c.8378G>A p.Gly2793Glu	3 (4,5)	5	Y
Sep-18	BRCA2	c.8524C>T p.Arg2842Cys	3 (4,5)	5 RP	Y
Oct-18	MLH1	c.1676T>G p.Leu559Arg	3 (4,5)	4	Y
Nov-18	MSH2	c.1807G>A p.Asp603Asn	4 (3,5)	4	Y
Jan-19	MLH1	c.440G>T p.Gly147Val	3 (4)	3	Y
Mar-19	MLH1	c.794G>A p.Arg265His	3	3	Y
Apr-19	BRCA1	c.5357T>C p.Leu1786Pro	3 (4)	4	Y
Apr-19	MLH1	c.1595G>A p.Gly532Asp	3 (4,5)	4	Y
May-19	APC	c.2497A>C p.Ser833Arg	3 (2)	3	Y
May-19	APC	c.6724A>G p.Ser2242Gly	3 (2)	2	Y
May-19	BMPR1A	c.1328G>A p.Arg443His	3 (4,5)	4	Y
May-19	BRCA1	c.5153-26 A>G	3 (4,5)	3	Y
May-19	BRCA2	c.520C>T p.Arg174Cys	3 (4)	3	Y
Sep-19	TP53	c.799C>T p.Arg267Trp	3 (4,5)	4	Y
Oct-19	TP53	c.1141A>G p.Lys381Glu	3 (4)	3	Y
Oct-19	TP53	c.322G>C p.Gly108Arg	4 (3,5)	3	Y
Oct-19	TP53	c.472 C>T p.Arg158Cys	3	3	Y
Oct-19	TP53	c.794T>A p.Leu265Gln	3 (4,5)	4	Y
Oct-19	TP53	c.560-14_560-13delCT	3 (4,5)	3	Y
Jan-20	TP53	c.453-455delCCC p.Pro153del	3 (4,5)	3	Y